

The development of asthma in children infected with *Chlamydia pneumoniae* is dependent on the modifying effect of mannose-binding lectin

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Background: Although several studies found associations between infection with *Chlamydia pneumoniae* and asthma, these were mainly restricted to the exacerbation of the symptoms in adults with known asthma. Data about the role of *C pneumoniae* in the initiation and development of asthma in children are controversial.

Objective: We investigated the role of *C pneumoniae* infection in 139 children with asthma, comparing them with 174 healthy control subjects. Furthermore, we studied the modifying effect of mannose-binding lectin (MBL) variant alleles on the susceptibility to asthma in children infected with *C pneumoniae*.

Methods: *C pneumoniae*-specific antibodies were measured by means of ELISA, and MBL genotypes were determined by means of PCR-RFLP.

Results: There were no significant differences in the percentage of children with positive results for *C pneumoniae*-specific antibodies between patients and control subjects. Among asthmatic children carrying variant MBL alleles, there were significantly more patients with positive results for *C pneumoniae*-specific IgG than among control children with variant MBL genotypes (63.7% vs 40.7% of asthmatic vs control children, respectively; odds ratio adjusted for age and sex, 2.21; 95% CI, 1.10-4.41; $P = .02$). Infected children with variant MBL alleles were found to have a higher risk of asthma development than infected children with normal MBL genotype. This risk was especially high in children with chronic or recurrent infection (positive results for both IgA and IgG; adjusted odds ratio, 5.38; 95% CI, 1.75-14.36; $P = .01$), but no increased risk was seen in children with current *C pneumoniae* infection (positive results for IgM).

Conclusion: This study indicates the important role of variant MBL alleles in the susceptibility to asthma in children infected with *C pneumoniae*. (J Allergy Clin Immunol 2003;112:729-34.)

Key words: Childhood asthma, mannose-binding lectin, polymorphism, bacterial infection, *Chlamydia pneumoniae*

Abbreviation used

MBL: Mannose-binding lectin

Chlamydia pneumoniae is a common respiratory pathogen, and although many infections are believed to be asymptomatic, a growing body of evidence implicates an association between *C pneumoniae* infection and asthma in some individuals.¹ The first association was described by Hahn et al,² who reported wheezing in 9 of 19 adult patients with serologic evidence of current or recent *C pneumoniae* infection. In another study 11% of asthmatic and 5% of control children were found to have positive cultures for *C pneumoniae*.³ Although some results were controversial,⁴ several studies confirmed these findings.^{5,6} However, if during their lifetime almost all individuals have been infected with *C pneumoniae*, why do some experience asthmatic symptoms and others do not? As several data indicate, genetic factors must play a role in the susceptibility to the disease and influence the lower respiratory response to *C pneumoniae* infection.

Mannose-binding lectin (MBL) is a complement-activating innate immune defense serum protein that binds to mannose and acetylglucosamine sugar groups on different microorganisms.⁷ Recent findings indicate that MBL inhibits infection of HeLa cells by different *Chlamydia* species, suggesting that MBL participates in the protection against *C pneumoniae*.⁸ MBL deficiency and low levels of serum MBL are strongly associated with the presence of variant MBL genes encoding 3 different structural variants of the MBL polypeptide.^{9,10} Single-nucleotide substitutions in codon 54 (allele B) and codon 57 (allele C) both result in the disruption of the repeated Gly-Xaa-Yaa structure of the collagenous triple helix by substituting the essential glycine residue with aspartic acid and glutamic acid, respectively. A nucleotide substitution in codon 52 (allele D) causes exchange of an arginine residue with a cysteine. All 3 variants are believed to prevent the assembly of MBL subunits into the basic trimer structure, thereby reducing the amount of functional MBL subunits in heterozygous individuals. These MBL variant alleles have been found to be associated with an increased risk of respiratory infections, especially during early childhood.^{11,12}

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TABLE I. Clinical and biologic characteristics of the asthmatic patients

Characteristics	All asthmatic patients
No.	139
Age, y (range, 3-18 y)	10.1 ± 4.1
Age at first diagnosis, y (range, 0.5-9 y)	3.7 ± 2.3
Sex, boy/girl	79/60
Atopy, %	78.4
<i>C pneumoniae</i> positive	55.1
Severity of asthma*	
Intermittent, %	38.8
Mild, %	46.1
Moderate, %	15.1
Severe persistent, %	0

*Asthma severity is classified according to Global Initiative for Asthma guidelines¹⁴ modified for children.¹⁵

On the basis of these observations, we investigated the role of *C pneumoniae* infection in children with asthma, comparing them with healthy control subjects. Furthermore, we studied the modifying effect of *MBL* variant alleles on the susceptibility to asthma in children infected with *C pneumoniae*.

METHODS

Subjects

One hundred thirty-nine asthmatic children (79 boys and 60 girls; age range, 3-18 years; mean age, 10.5 years [SD 4.1]) and 174 healthy children (95 boys and 79 girls; age range, 3-18 years; mean age, 11.5 years [SD 3.9]) were enrolled in the study. All patients were from Budapest.

The asthmatic children attended the Allergic Outpatient Consultation of the Budai Children's Hospital. All the asthmatic children had specialist-diagnosed asthma with the following characteristics: (1) recurrent breathlessness and expiratory dyspnea requiring treatment; (2) physician-diagnosed wheeze; and (3) reversibility of the wheezing and dyspnea by means of bronchodilator treatment measured as FEV₁ by using a spirometer (Piston).¹³

All the asthmatic patients (or their parents) were instructed to record accurately their symptoms, treatment, and peak expiratory flow (twice daily, once in the evening and once in the morning) for 2 weeks. If the patient was younger than 5 years of age, the determination of lung function test results (peak expiratory flow or FEV₁) is usually not possible. In that case the diagnosis of the disease was made according to the other symptoms. Asthma severity was classified according to Global Initiative for Asthma guidelines^{14,15}; a detailed description of our classification of asthma severity can be found in Szalai et al.¹⁵ Some clinical and biologic characteristics of the asthmatic patients are presented in Table I.

The control children were randomly selected from outpatients from the Orthopaedic Department in the Budai Children's Hospital. Subjects in the control group had mild musculoskeletal alterations (eg, pes planus or scoliosis), showed no symptoms of asthma or infection, and required no medication. Stratification by age and sex was used to match approximately the age and sex distribution of the control subjects with that of the patients. Informed parental consent was obtained for each patient, and the study was approved by an institutional review committee.

Laboratory analysis

Total genomic DNA was extracted from white blood cells by using the method of Miller et al.¹⁶

The *MBL* polymorphisms were determined similarly, as described elsewhere.¹⁷ Briefly, PCR was used to amplify a 119-bp region of exon 1 containing codons 52, 54, and 57. The upstream primer was a mutated oligonucleotide (5'-CAT CAA CGG CTT CCC AGG CAA AGA CGC G-3') that introduced a new restriction enzyme cut site in the presence of the codon 52 mutation. The downstream primer was 5'-CAG GCA GTT TCC TCT GGA AGG-3'. PCR was performed in a reaction volume of 70 μL in a Biometra TRIO-Thermoblock. The PCR reactions were 95°C for 4 minutes; 35 cycles of 95°C for 30 seconds, 63°C for 1 minute, and 72°C for 30 seconds; and 72°C for 5 minutes. PCR products were digested in separate tubes with the restriction enzymes *Ban* I, *Mbo* II, and *Mlu* I (New England Biolabs), followed by separation in a 4% agarose gel and ethidium bromide staining. The presence of mutation in codon 52 introduces the *Mlu* I cut site (allele *D*) and produces 2 fragments (95 and 24 bp), as opposed to a single 119-bp fragment in the wild-type allele. The presence of mutation in codon 54 (allele *B*) eliminates the restriction site for *Ban* I present in wild-type DNA, and therefore the PCR product runs as a single 119-bp band as opposed to 2 bands (84 and 35 bp in wild-type DNA). The presence of mutation in codon 57 introduces the *Mbo* II restriction site (allele *C*), and therefore the PCR product is seen as 2 bands (79 and 40 bp) as opposed to a single 119-bp fragment in wild-type DNA. The normal allele is designated A, and the common designation of the variant alleles is 0.¹⁸

C pneumoniae-specific IgA, IgG, and IgM were determined from serum used (Sero CP-IgA, IgG, IgM protein ELISA; Savyon Diagnostic Ltd), according to the manufacturer's instruction. Serum was designated as positive if the cutoff index was greater than 1.1. Patients with positive results for *Chlamydia trachomatis* or *Chlamydia psittaci* were excluded from the study.

Statistical methods

Allele frequencies were calculated by means of allele counting. Data were analyzed by using MedCalc 5.0, SPSS 11.0, and Arlequin 1.1 programs. Hardy-Weinberg equilibrium was tested by using a χ² goodness-of-fit test. χ² Tests were used to test for differences in allele distribution between the groups. CIs were calculated at the 95% level. Two-tailed *P* values of less than .05 were considered statistically significant. It has earlier been suggested that the response to the *C pneumoniae* infection is influenced by sex.⁶ Furthermore, age, especially in childhood, might also have a substantial effect on the immune response. The results were adjusted for age and sex by means of multiple logistic regression analysis to minimize the effect of these 2 potentially confounding factors.

RESULTS

Table II shows the number and percentage of children with positive results for *C pneumoniae*-specific antibodies in the 2 groups. There were no significant differences in the proportion of patients with positive results for any *C pneumoniae*-specific antibodies between asthmatic and control children. Furthermore, there were no differences between sexes in the infection status in any groups of children (data not shown).

By definition, IgG positivity indicates that the probands have had *C pneumoniae* infection in the past, and IgM positivity indicates a current infection. The presence of short-lived specific IgA antibodies to *C*

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TABLE II. Number and percentage of asthmatic and control children with positive results for *C pneumoniae*-specific antibodies

	Asthmatic patients				Control patients				P value*			
	IgA	IgG	IgM	IgG + IgA	IgA	IgG	IgM	IgG + IgA	IgA	IgG	IgM	IgG + IgA
No. of patients	137	135	138	134	174	173	174	173	1.0	.6	.4	.9
Positive results for <i>C pneumoniae</i> , n (%)	43 (31.4)	59 (43.7)	28 (20.3)	27 (20.1)	56 (32.2)	82 (47.4)	43 (24.7)	37 (21.3)				

*Asthmatic patients versus control subjects (P value was calculated by using the χ^2 test).

pneumoniae can be associated with both acute and chronic infection (persistent levels of IgA). Chronic or recurrent infection with *C pneumoniae* might be identified when IgG positivity is combined with IgA positivity. According to this latter definition, 27 (20.1%) asthmatic patients and 34 (21.3%) control subjects had chronic or recurrent *C pneumoniae* infection among our patients. The 2 groups did not differ statistically in this respect either (Table II).

The occurrence of the *MBL* genotypes and frequencies of the different *MBL* alleles in asthmatic and control children are presented in Table III. There were no significant differences in the prevalence of any alleles between patients and control subjects. Furthermore, no significant difference between *C pneumoniae*-seropositive and *C pneumoniae*-seronegative individuals in the frequency of *MBL* variant alleles was revealed: frequencies of 0 allele in children with IgG positivity, 29.6%, IgG negativity, 23.8% ($P = .1$); IgA positivity, 28.3%; IgA negativity, 25.0% ($P = .5$); IgM positivity, 28.7%; IgM negativity, 26.0% ($P = .6$). The allelic distributions were in Hardy-Weinberg equilibrium.

To evaluate the modifying effect of *MBL* variant alleles on the susceptibility to asthma in *C pneumoniae* infection, children were stratified according to *MBL* alleles, and the asthmatic and control children were compared on the basis of their infection status (Table IV).

Among asthmatic children heterozygous and homozygous for the variant (A/0 and 0/0) *MBL* alleles, there were significantly more patients with positive results for *C pneumoniae*-specific IgG than among control children with variant *MBL* genotypes (63.7% vs 40.7% of asthmatic vs control children, respectively; $P = .008$; odds ratio [OR], 2.61; 95% CI, 1.32-5.15; Table IV). The results remained significant after adjusting for age and sex ($P = .02$; OR, 2.21; 95% CI, 1.10-4.41). These results indicate that *C pneumoniae*-specific IgG positivity was associated with asthma when only children carrying *MBL* variant 0 alleles were considered.

In contrast, comparing asthmatic children homozygous for the normal allele (A/A) with control children with identical *MBL* genotypes, there were significantly fewer asthmatic patients with positive results for *C pneumoniae*-specific IgG than control children, even after adjustment for age and sex (27.7% vs 49.4% of asthmatic vs control children, respectively; $P = .003$; OR, 0.33; 95% CI, 0.16-0.68). This suggests that homozygosity for the normal *MBL* variant might have a protective effect against asthma in infected children.

TABLE III. Occurrence of the *MBL* genotypes and frequencies of the different alleles in asthmatic and control children

<i>MBL</i> genotype*	Patients	Control subjects
A/A	70	91
A/B	37	39
A/C	5	8
A/D	19	18
B/B	2	2
B/C	2	2
B/D	3	5
C/C	1	0
C/D	0	0
D/D	0	0
Sum	139	165
B allele frequency (%)	16.5	15.2
C allele frequency (%)	3.2	3.0
D allele frequency (%)	7.9	7.0
0 allele frequency (%)	27.7	25.2

The frequencies of the variant alleles did not differ between the 2 groups: $P = .7$; OR, 1.14; 95% CI, 0.79-1.64 (χ^2 test).
A, Normal *MBL* allele; B, codon 54 variant allele; C, codon 57 variant allele; D, codon 52 variant allele; 0, common designation of the variant alleles (B+C+D).

There were no significant differences between asthmatic and control children when *MBL* genotypes and *C pneumoniae*-specific IgA and IgM were considered (Table IV), although after adjusting for age and sex, the differences were near to the level of significance ($P = .06$) in the case of IgA, with similar tendencies in the case of IgG. The differences were not significant when children with combined IgA and IgG positivity were considered.

The prevalence of different *MBL* genotypes in subjects with positive results for *C pneumoniae*-specific antibodies were compared in asthmatic patients and control subjects to further evaluate the role of *MBL* genotypes in susceptibility to asthma among children infected with *C pneumoniae* (Table V).

The distribution of genotypes differed significantly between asthmatic and control children with positive results for *C pneumoniae*-specific IgA, IgG, or both. In each case the proportion of children with variant *MBL* alleles was higher in asthmatic patients and lower in healthy control subjects, comparing results with those in children with the normal genotype. The OR was the highest when children with positive results for both *C pneumoniae*-specific IgA and IgG were considered

TABLE IV. Logistic regression analysis for the association between the prevalence of different *C pneumoniae*-specific antibodies and asthma in children according to *MBL* genotypes

<i>C pneumoniae</i> status	Patients, n (%)	Control subjects, n (%)	OR (95% CI)	<i>P</i> value	Adjusted* OR (95% CI)	<i>P</i> value*
Subjects homozygous for the normal (A/A) <i>MBL</i> alleles						
IgG positive	18 (27.7)	44 (49.4)	0.39 (0.20-0.78)	.007	0.33 (0.16-0.68)	.003
IgG negative	47 (72.3)	45 (50.6)				
Subjects heterozygous and homozygous for the variant (A/O and O/O) <i>MBL</i> alleles						
IgG positive	44 (63.7)	29 (40.3)	2.61 (1.32-5.15)	.008	2.21 (1.10-4.41)	.02
IgG negative	25 (36.2)	43 (59.7)				
Subjects homozygous for the normal (A/A) <i>MBL</i> alleles						
IgA positive	13 (20.0)	33 (35.5)	0.45 (0.22-0.95)	.05	0.46 (0.21-1.08)	.06
IgA negative	52 (80.0)	60 (64.5)				
Subjects heterozygous and homozygous for the variant (A/O and O/O) <i>MBL</i> alleles						
IgA positive	27 (39.1)	17 (25.4)	1.90 (0.91-3.93)	.1	2.04 (0.95-4.18)	.06
IgA negative	42 (60.9)	50 (74.6)				
Subjects homozygous for the normal (A/A) <i>MBL</i> alleles						
IgM positive	10 (15.2)	24 (26.7)	0.49 (0.22-1.11)	.1	0.51 (0.22-1.18)	.16
IgM negative	56 (84.8)	66 (73.3)				
Subjects heterozygous and homozygous for the variant (A/O and O/O) <i>MBL</i> alleles						
IgM positive	18 (26.1)	16 (22.2)	1.23 (0.57-2.68)	.7	1.12 (0.56-2.75)	.58
IgM negative	51 (73.9)	56 (77.8)				
Subjects homozygous for the normal (A/A) <i>MBL</i> alleles						
IgG + IgA positive	9 (13.8)	26 (29.2)	0.39 (0.17-0.90)	.04	0.45 (0.22-1.05)	.06
IgG + IgA negative	56 (86.2)	63 (70.8)				
Subjects heterozygous and homozygous for the variant (A/O and O/O) <i>MBL</i> alleles						
IgG + IgA positive	17 (24.6)	9 (12.7)	2.25 (0.93-5.47)	.1	2.18 (0.96-5.34)	.07
IgG + IgA negative	52 (75.4)	62 (87.3)				

*Adjusted for age and sex.

TABLE V. Logistic regression analysis for the association between the prevalence of different *MBL* genotypes and asthma in children with positive results for different *C pneumoniae*-specific antibodies

<i>C pneumoniae</i> status		<i>MBL</i> genotypes		OR (95% CI)	<i>P</i> value	Adjusted OR* (95% CI)	<i>P</i> value*
		A/O + O/O	A/A				
IgA positive	Asthmatic patients	27 (20.1)	13 (9.7)	4.03 (1.71-9.75)	.003	3.95 (1.52-8.78)	.005
	Control subjects	17 (10.6)	33 (20.6)				
IgG positive	Asthmatic patients	44 (32.8)	18 (13.4)	3.71 (1.80-7.63)	.001	3.55 (1.62-6.93)	.005
	Control subjects	29 (18.0)	44 (27.3)				
IgM positive	Asthmatic patients	18 (13.3)	10 (7.4)	2.70 (0.99-7.33)	.09	2.41 (0.91-6.88)	.1
	Control subjects	16 (9.9)	24 (14.8)				
IgG + IgA positive	Asthmatic patients	17 (12.7)	9 (6.7)	5.46 (1.80-16.52)	.005	5.38 (1.75-14.36)	.01
	Control subjects	9 (5.6)	26 (16.3)				

Values are given as numbers (percentages).

*Adjusted for age and sex.

(adjusted OR, 5.38; 95% CI, 1.75-14.36; *P* = .01). Children with positive results for *C pneumoniae*-specific IgM did not differ in this respect. These results indicate that children with variant *MBL* alleles and positive results for *C pneumoniae*-specific IgA, IgG, or both have considerably higher risk of asthma development than similarly infected children with normal *MBL* genotype.

Several previous reports found a link between the exacerbation of the symptoms of asthma and chronic *C pneumoniae* infection.^{2,19,20} We used the recommendations for classification of asthma severity of the Global Initiative for Asthma guidelines.^{14,15} Among the 139 asthmatic patients, 54 (38.8%) had intermittent, 64 (46.1%) had mild, and 21 (15.1%) had moderate asthma.

Asthma, rhinitis, other
respiratory diseases

There were no patients with severe persistent asthma in this population (Table 1). To assess the effect of chronic *C pneumoniae* infection on the severity of asthma in this population, we compared the prevalence of children with IgG plus IgA positivity in groups with intermittent and moderate asthma. Among the 54 children with intermittent asthma, 12 (22.2%) had positive results for IgG and IgA, whereas among the 21 children with moderate asthma, 5 (23.8%) patients had positive results for these 2 *C pneumoniae*-specific antibodies. The 2 proportions do not differ from each other significantly ($P = .9$). The 2 groups did not show a significant difference either when *MBL* variant alleles were considered. The prevalence of children with variant *MBL* alleles and positive results for *C pneumoniae* IgG and IgA were 11.1% (6/54) and 9.5% (2/21) in the intermittent and moderate groups, respectively ($P = .8$). These data indicate that in our patients chronic or recurrent *C pneumoniae* infection does not play a significant role in the severity of asthmatic symptoms, not even when *MBL* variant alleles are considered.

Among the asthmatic patients, 109 (78.4%) were atopic. The *C pneumoniae* infection, the *MBL* variant alleles, and their combinations were not associated with atopy ($P > .05$).

DISCUSSION

This study supports the association between *C pneumoniae* infection and asthma; however, the association was dependent on variation in the *MBL* gene. Significantly increased risk for development of asthma was seen in infected children who carried at least one variant *MBL* allele. However, this association was restricted to children with past *C pneumoniae* infection (positive for IgG). Furthermore, infected children with variant *MBL* alleles were found to have considerably higher risk of development of asthma than infected children with the normal *MBL* genotype. This risk was especially high in children with chronic or recurrent infection (positive results for both IgA and IgG), but no increased risk was seen in children with current *C pneumoniae* infection (positive results for IgM).

A causal association between *C pneumoniae* and asthma is biologically plausible on the basis of the observations that asthma is a chronic inflammatory disease of the airways and that *Chlamydia* species are known to produce chronic inflammatory damage in target organs. Although several studies found associations between infection with *C pneumoniae* and asthma, these were mainly restricted to the exacerbation of the symptoms in adults with known asthma.^{2,19,20} Data about the role of *C pneumoniae* in the initiation and development of asthma in children were controversial and not conclusively proven.¹⁹ In our study there was also no direct connection between the disease and *C pneumoniae* because the prevalence of the infection was practically the same in children with asthma and in healthy control subjects. But the difference between the 2 groups became significant when the subjects were stratified according to their *MBL*

genotypes and *C pneumoniae*-specific IgG was considered. According to these results, *C pneumoniae* infection might lead to the development of asthma in genetically predisposed children carrying *MBL* variant alleles. Naturally, a reverse scenario is also feasible. Asthmatic children with variant *MBL* alleles are prone to development of a chronic *C pneumoniae* infection, which could then propagate the asthma process. Although our results that in this population chronic or recurrent *C pneumoniae* infection did not play a significant role in the severity of asthmatic symptoms, not even when *MBL* variant alleles were considered, did not support this hypothesis, prospective studies are needed to determine the exact role of this genotype-environment interaction in asthma.

MBL deficiencies have been implicated in *C pneumoniae* infection because the bacteria carry the sugar structures relevant for *MBL* binding.⁸ Moreover, *MBL* inhibits the uptake of the bacteria to target cells in vitro.⁸ We did not observe a direct protective effect of *MBL* against *C pneumoniae* per se, which is not unexpected because the seroprevalence in healthy control subjects exceeds 50%, but infected individuals homozygous for the normal allele (*A/A*) were protected against the development of asthma. *MBL* is not present in the normal lung, which explains the lack of protection against *C pneumoniae* infection, but in animal studies it appeared in lung lavage fluid 3 days after experimental infection with influenza virus.²¹ In addition, *MBL* was detected in lung expectoration from some patients with cystic fibrosis.¹² Considering that individuals with the *A/A* genotype have on average 10-fold higher *MBL* serum concentrations than heterozygous variant allele carriers and homozygous carriers have no serum *MBL* at all,¹² the role of *MBL* in killing or opsonization of *C pneumoniae* in the lung might be more pronounced in normal allele carriers, which can lead to this significant alteration in the outcome of infection. Alternatively, *MBL* might act to modulate the severity of the infection, rather than protect against it. Experimental studies support such a view in which it has been shown that *MBL* might modulate cytokine responses.²² Furthermore, *MBL* deficiency has been shown in vivo to be implicated in several chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, or atherosclerosis.^{18,23,24} Because asthma is also a chronic inflammatory disease, it can be suggested that *MBL* deficiency might have important roles either directly or indirectly in several chronic inflammatory diseases. In addition, in the case of atherosclerosis, *MBL* deficiency has also been found to modulate the progression of *C pneumoniae*.¹⁸ Similar to our findings, only those patients with positive results for *C pneumoniae*-specific IgG and at least one defective *MBL* allele had a significantly higher risk of development of the disease. The coincidence of results of these 2 independent studies further strengthens the evidence for involvement of *MBL* in the immune response to *C pneumoniae* infection.

In our study we did not find an association between current infection with *C pneumoniae* (IgM positivity), *MBL* deficiency, and asthma, indicating that there must be a time interval between infection and the development

of asthmatic symptoms. This is in good correlation with previous results that only chronic or past infection was found to be associated with asthma or wheezing.^{2,19,20,25} This finding might also have a therapeutic implication: elimination of *C pneumoniae* with antibiotics in freshly infected patients might prevent the development of asthma in genetically affected individuals (eg, with MBL deficiency). However, considering the high cost of the available tests and that IgM antibodies are usually lost within 2 to 6 months, regular screening for *C pneumoniae*-specific antibodies is not feasible, not even for individuals with MBL deficiency.

These results are in good correlation with the findings of some recent reports that investigate the role of variations in the otherwise conservative genes in the innate immunity.^{26,27} The effects of these variations are usually modest and insufficient to cause disease when taken individually. However, they might induce significant phenotypic differences when they become involved in gene-by-gene interaction, gene-by-environment interaction, or both. Our finding that the outcome of *Chlamydia* species infection seems to be opposite in individuals who are wild type for the normal *MBL* allele or carriers for the variant alleles is a further example of a nonlinear gene-by-environment interaction.

In conclusion, this is the first report illustrating an important association between MBL deficiency, *C pneumoniae* infection, and the development of asthma. According to the present findings, there is an increased risk for development of asthma in children with positive results for *C pneumoniae*-specific IgG and who carry at least one variant *MBL* allele. Furthermore, infected children with variant *MBL* alleles were found to have considerably higher risk of development of asthma than infected children with the normal *MBL* genotype. This risk was especially high in children with chronic or recurrent infection, but no increased risk was seen in children with current *C pneumoniae* infection. However, the relative small size and lack of supportive biologic data make it necessary that these observations are confirmed in additional prospective studies before definitive conclusions can be drawn. If confirmed, these results suggest that genotyping individuals for *MBL* gene polymorphisms might be useful in predicting asthma risk in children infected with *C pneumoniae* and identifying those who can benefit more likely from the antibiotic therapy.

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